C=the number of units of procaine penicillin per milliliter as determined in paragraph (a)(3)(iii) of this section.
F=the appropriate dilution factor depending on the dilutions made in the preparation of the solution for assay.

The content of buffered crystalline penicillin is satisfactory if the batch contains 85 percent of the number of units per milliliter that it is represented to contain.

(4) *Benzathine penicillin content.* The sum of the procaine penicillin content determined under paragraph (a)(2) or (6) of this section and the buffered crystalline penicillin content determined under paragraph (a)(3) of this section, subtracted from the total potency determined in paragraph (a)(1) or (5) of this section, represents the benzathine penicillin G content. The benzathine penicillin G content is satisfactory if it is not less than 85 percent of the number of units that it is represented to contain.

(5) *Total potency of a single-dose container.* Wash out the material remaining in the volumetric flask referred to in paragraph (a)(3)(i)(*a*) of this section, or combine the contents remaining in the 50-milliliter volumetric flask and in the centrifuge tube referred to in paragraph (a)(3)(i)(*b*) of this section. Dissolve the material by adding 10 milliliters of 1 *N* NaOH for each 300,000 units of benzathine penicillin and allow to stand 15 minutes. Add 1 milliliter of 1.2 *N* HCl for each milliliter of 1 *N* NaOH and then dilute with distilled water to give a concentration of approximately 2,000 units per milliliter. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and then titrate with 0.01 *N* sodium thiosulfate as directed in §440.80a (b)(5)(iv)(*a*) of this chapter. For the blank determination prepare a separate sample as directed in paragraph (a)(3)(i) (*a*) or (*b*) of this section and in the first sentence of this paragraph (a)(5), then dilute with 1 percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. The total potency of the one-dose container is equal to the sum of the number of units found in this assay (units per milliliter×volume) and the number of units found (units per milliliter ×volume) in the solution for assay in paragraph (a)(3)(ii) of this section.

(6) *Procaine penicillin content of a single-dose container.* Make suitable dilutions of the NaOH-inactivated solution prepared in paragraph (a)(5) of this section to obtain approximately 60 units of procaine penicillin per milliliter. Determine the procaine penicillin content (units per milliliter× volume) of this solution by the colorimetric procedure described under §436.503(b)(3). To this value add per procaine penicillin content (unit per milliliter×volume) of the solution for assay, as found in paragraph (a)(3)(iii) of this section, to obtain the procaine penicillin content of the one-dose container. The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(b) *Sterility.* Proceed as directed in §436.20, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(c) *Pyrogens.* Proceed as directed in §440.55a(b)(4) of this chapter.

(d) *Toxicity.* Proceed as directed in §440.55a(b)(3) of this chapter.

(e) *Moisture.* Proceed as directed in §440.74a(b)(5) of this chapter.

(f) *pH.* Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the product is reconstituted as directed in the labeling.

§436.508 Penicillin - bacitracin - neomycin ointment; penicillin-bacitracin-neomycin in oil.

(a) *Potency*—(1) *Penicillin content; bacitracin content.* Proceed as directed in §436.504(a).

(2) *Neomycin content.* Proceed as directed in §448.510d(b)(1)(ii) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.509 Procaine penicillin-streptomycin-polymyxin in oil; procaine penicillin-dihydrostreptomycin-polymyxin in oil; procaine penicillin-streptomycin-polymyxin ointment; procaine penicillin - dihydrostreptomycin - polymyxin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 544.373(b)(1)(i) of this chapter, except inactivate the penicillin in the combined extractives with sufficient penicillinase at 37° C. for 30 minutes. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as a standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(4) *Polymyxin content.* Proceed as directed in § 444.170a(b)(2)(i) of this chapter, with the following exceptions:

(i) In lieu of the directions for the preparation of the sample described in § 444.170a(b)(2)(i)(*g*) of this chapter, prepare the sample by one of the following techniques:

(*a*) *Extraction.* Place a convenient-sized representative quantity of the sample in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer (pH 6.0) and shake. Remove the buffer layer and repeat the extraction with 25-milliliter portions of buffer at least three times and any additional times that may be necessary to insure complete extraction of the antibiotic. Combine the extractives. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes. Make the proper estimated dilutions in 10-percent potassium phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(*b*) *Blending.* Place a convenient-sized representative quantity of the sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 1-percent phosphate buffer (pH 6.0) to give a final volume of 200 milliliters. If the sample consists of substantially more than 1 gram, use sufficient buffer to give a final volume of 500 milliliters. If the concentration of polymyxin in the blend is less than 200 units per milliliter, 10-percent phosphate buffer (pH 6.0) should be used in lieu of 1-percent phosphate buffer (pH 6.0). Using a high-speed blender, blend the mixture for 2 minutes. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes and make the proper estimated dilutions in 10-percent phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(ii) The standard curve is prepared in the following concentrations: 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter in 10-percent potassium phosphate buffer, pH 6.0. The 10 units per milliliter concentration is used as the reference point. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

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§ 436.510 Penicillin-streptomycin-erythromycin ointment; penicillin-dihydro-streptomycin-erythromycin ointment.

(a) *Potency*—(1) *Penicillin content.* Obtain the weight of the content of a syringe by weighing before and after ejecting the content into a beaker. Stir until homogeneous. Remove a representative sample (usually approximately 1.0 gram, accurately weighed) and place in a separatory funnel containing 50 milliliters of peroxide-free